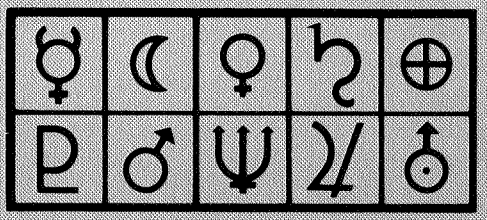

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DEPOSITION OF NUTRIENTS TO SURFACES BY RODAC PLATES

(Part II of Microbiological Studies Relating to Clean Environments)

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ABSTRACT

The deposition of nutrient residues onto surfaces following impressions made with Rodac plates has been observed, photographed, and quantitated. In the experiments performed, a medium residue between 20 and 50 micrograms in weight was deposited from Rodac plates onto stainless steel surfaces. Such residues were shown to be adequate to support microbial growth when such surfaces were contaminated and incubated under ideal conditions of humidity and temperature.

*Microbiologist, Department of Biology, University of New Mexico, and Sandia Laboratory Consultant.

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DEPOSITION OF NUTRIENTS TO SURFACES BY RODAC PLATES

Introduction

The development of the laminar-flow clean room[1] has made it possible to achieve sterile or near-sterile intramural air in clean rooms. Since it is now possible to attain environments virtually free of all particulate contamination, it becomes important to be able to monitor surfaces within the clean work areas in order to (1) discover potential sources of particulate contamination of intramural air, (2) monitor surfaces of equipment brought into clean rooms, and (3) monitor equipment as it is processed in the clean room.

The efficiency of laminar-flow systems has forced available particulate monitoring technology (airborne and surface, viable and non-viable) to the limit of present capabilities. Available techniques for monitoring viable contamination on surfaces consist of (1) rolling a sterile moist swab over the surface, followed by an appropriate assay of the viable particles picked up by the swab,[2, 3] or (2) using agar impressions.[4, 5, 6] Of these, the use of agar impressions is the more efficient technique where it can be applied[7] and, in the past few years, the Rodac plate[8] has been widely used in installations where microbial monitoring of surfaces has been desired, such as in hospital operating theaters.

Since it is necessary to anticipate potential requirements and to develop methods and techniques suitable for satisfying these requirements, it was considered advisable to evaluate the Rodac plate technique for potential use in monitoring viable microbial surface contamination. For example, it seemed reasonable to assume that when Rodac impressions are taken from surfaces some small amount of residual material would be deposited onto the surface by the plate itself.

The present investigation was undertaken to determine (1) whether Rodac plates do leave residual nutrient materials after impressions are taken, (2) the quantity of such materials, and (3) whether the residual material would support the growth of selected species of bacteria under ideal conditions of temperature and humidity.

Materials and Methods

Two methods were applied to determine whether nutrient medium residues would be deposited onto surfaces following impressions by Rodac plates. The first technique was used in an effort to provide visual and photographic evidence for such medium deposition, and the second technique was used in an attempt to quantitate the amount of medium deposited.

For visual and photographic evidence, Rodac plates were prepared with 15. 5 ml of trypticase soy agar (Baltimore Biological Laboratories) to which had been added 0. 5 percent fluorescein (Uranine, Schultze No. 585 water soluble, National Aniline Chemical Co.). The plates were stored at 4°C until

ready for use. Impressions were made with the Rodac plate on burnished stainless steel plates in the recommended manner. The fluorescein medium under ultraviolet light made possible the photographic records obtained with a K-2 filter and Ektachrome film in a plate film copy camera.

In order to determine the quantity of nutrient medium deposited on the surface by Rodac plate impressions, Rodac plates were prepared with trypticase soy agar and distilled water. The plates were poured, allowed to solidify, and dried for approximately 1 hour. A Rodac plate was then pressed onto the bottom of a stainless steel beaker. Ten milliliters of distilled water were added to the beaker, the beaker was then sonified in a Turco high-power tank sonifier, and the water was assayed in the solvent purity meter.[9] Readings of 0. 95 from the impression of one Rodac plate and 1. 10 from four such impressions superimposed upon one another, compared with a reading of 0. 75 for the control beaker (which had no impressions), indicated that assayable quantities of medium were being deposited onto the surfaces.

The solvent purity meter was calibrated and a standard curve prepared in the following manner: 1. 0001 grams of trypticase soy agar was added to 25. 0 ml of distilled water. To ensure that only the nutritive materials would be dissolved, and the agar left in suspension, the dilution was left unheated and was then assayed in the solvent purity meter. A calibration curve is shown in Figure 1, and calibration readings are given in Table I.

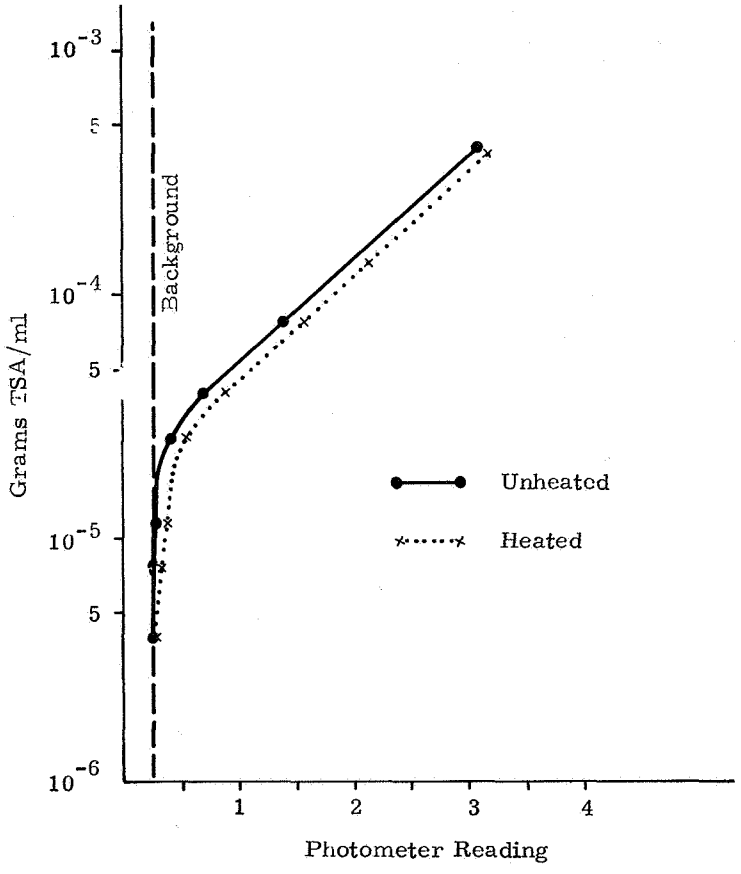

Figure 1. Solvent Purity Meter Calibration Curve for Unheated and Heated Trypticase Soy Agar in Distilled Water

A second calibration curve was prepared using 4. 0003 grams of trypticase soy agar added to 100 ml of distilled water and dissolved by heating. A 10^{-2} dilution was prepared from this medium, boiled, restored to volume, further diluted and assayed in the solvent purity meter. Resulting calibration readings are given in Table I and a related calibration curve is shown in Figure 1.

TABLE I

Solvent Purity Meter Calibration Readings, Using Unheated and Heated Trypticase Soy Agar

Dilution	Agar Unheated	Agar Heated
10^{-2}	3. 30	3. 10
33. 3 ml of 10^{-2} in 100 ml	2. 20	----
20. 0 ml of 10^{-2} in 100 ml	1. 60	1. 40
10. 0 ml of 10^{-2} in 100 ml	0. 90	0. 70
5. 0 ml of 10^{-2} in 100 ml	0. 50	0. 45
3. 0 ml of 10^{-2} in 100 ml	0. 35	0. 30
2. 0 ml of 10^{-2} in 100 ml	0. 30	0. 25
1. 0 ml of 10^{-2} in 100 ml	0. 25	0. 25
Deionized distilled water (background)	0. 25	0. 25

Following the calibration of the meter, Rodac impressions were made on the bottom of stainless steel beakers, 10 ml of distilled water added, the beaker sonified in the Turco tank sonifier, and the solution assayed in the solvent purity meter. Three trials were made, prepared, and treated in the following manner:

Trial A -- One impression made; Rodac surface slightly moist; impression not allowed to dry prior to adding 10 ml in distilled water.

Trial B -- One impression made; Rodac surface slightly moist; impression allowed to dry prior to adding 10 ml in distilled water.

Trial C -- One impression made; Rodac surface dry; impression allowed to dry prior to adding 10 ml in distilled water.

Following the above treatment, the beakers were sonified, readings were measured in the solvent purity meter, and the quantity of medium was obtained from the calibration curves.

Several experiments were performed in order to verify the assumption that any medium residual on surfaces would support microbial growth provided that the proper environmental conditions were present. Contamination of burnished stainless steel plates and glass slides was achieved in three ways:

1. Rodac plates were seeded with organisms prior to making impressions.
2. Impressions were made with Rodac plates, after which the surface was sprayed with the test organism, using a DeVilbiss No. 40 nebulizer.
3. The glass and stainless surfaces were sprayed with the test organism and allowed to air dry; impressions were made.

Control surfaces were sprayed with the test organism in an identical manner, but no Rodac impressions were made. These controls were incubated in the same moist chamber as the test surfaces.

Escherichia coli, Bacillus subtilis (B. globigii), and Serratia marcescens were used as test organisms. Following the indicated procedure, the contaminated glass slides and stainless steel plates were placed in a desiccator jar having a saturated atmosphere, and incubated for 3 days at 28°C. The Rodac plates were also incubated as a control on the ability of the medium to support the growth of the test organisms.

Stainless steel control and test surfaces were photographed at a magnification of 720 under vertical illumination.

Results and Discussion

Medium residue deposited on stainless steel plates was apparent when observed under ultraviolet illumination. Since fluorescein is fluorescent only when in solution, the rapid drying of the residue complicated photographic records; however, dry impression residues could be seen clearly even though they were no longer fluorescent. A typical medium residue obtained in such experiments is shown in Figure 2.

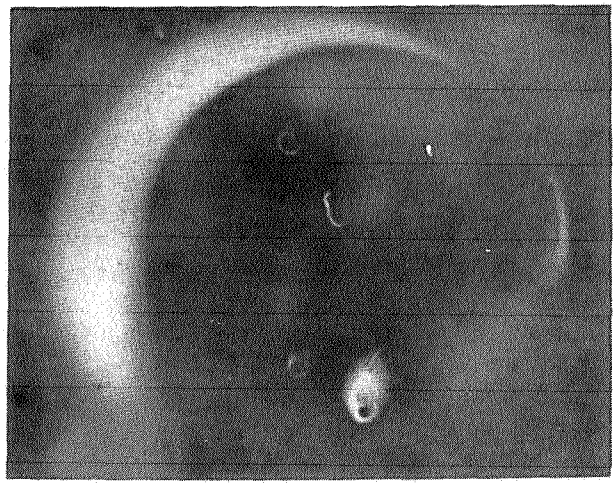

Figure 2. Typical Residue Deposited by Rodac Plate Impression, Using a Medium Containing Fluorescein and Illuminated With Ultraviolet Light

The results of the quantitative assay of residual medium are shown in Table II. It is apparent that significant amounts of medium are deposited on surfaces by Rodac impressions and, considering the size of bacteria and their mass, it may be inferred that the quantity is adequate to provide sufficient nutrient for organisms to proliferate.

TABLE II

Quantitation of Medium Residual From Rodac Impressions

Experiment	Purity Meter Reading	TSA Residual (mg)
A	1.00	0.046
B	0.60	0.026
C	0.50	0.02

To verify the assumption that residual nutrients were present in sufficient quantities to support bacterial growth under the proper environmental conditions, growth chamber experiments were performed. All control Rodac plates showed confluent growth of the test organisms, verifying the ability of the medium to support their growth. Growth of the test organisms was also readily observed on all of the test surfaces. Figure 3 shows a typical burnished steel surface which had been sprayed with a test organism (S. marcescens) but on which no Rodac impression was made. It can be seen that the organisms are present in very small numbers. This photograph should be compared with Figure 4, which shows a typical steel surface that was sprayed with the test organism after a Rodac impression had been made. The ring of confluent bacterial growth which follows the pattern of the deposit of medium residue is apparent.

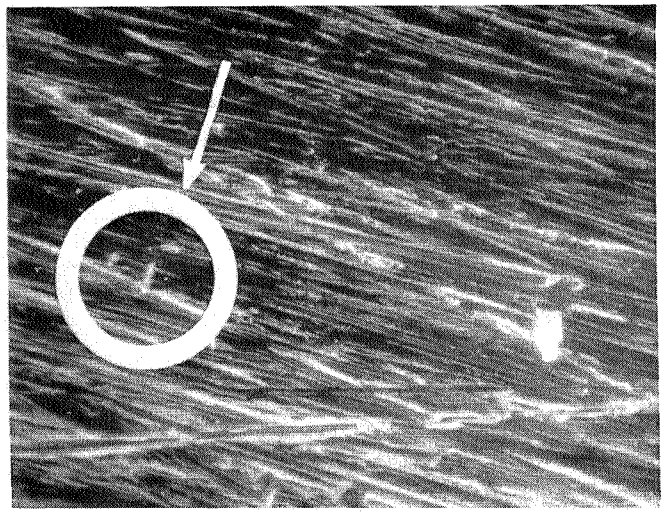

Figure 3. Burnished Stainless Steel Surface Sprayed With Serratia marcescens; No Rodac Impression Was Made; Incubated 3 Days in Saturated Atmosphere at 28°C. (x 720)

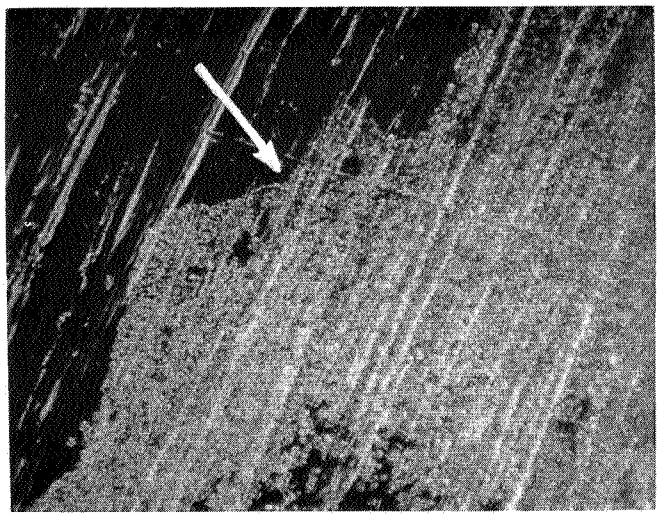

Figure 4. Burnished Stainless Steel Surface Sprayed With Serratia marcescens After a Rodac Impression Was Made; Incubated 3 days in a Saturated Atmosphere at 28°C. (x 720)

These results clearly show that Rodac plates do deposit residues of nutrient materials in sufficient quantity to support bacterial growth of the species tested under proper environmental conditions. No attempts were made to delineate the limits of temperature and humidity required to support bacterial growth on the surfaces so treated.

The results also show that while Rodac plates are an effective method for the monitoring of surfaces, any utilization of them for surface sampling in clean rooms should be accompanied by immediate cleansing procedures to remove any residual medium deposited by the impression. This has particular significance in applications in which microbial contamination must be reduced to an absolute minimum.

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